Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Veterinary Parasitology 126 (2004) 37–56
veterinary parasitology
www.elsevier.com/locate/vetpar

Review

Cryptosporidium: a water-borne zoonotic parasite

Ronald Fayer*

United States Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, USA

Abstract

Of 155 species of mammals reported to be infected with *Cryptosporidium parvum* or *C. parvum*-like organisms most animals are found in the Orders Artiodactyla, Primates, and Rodentia. Because *Cryptosporidium* from most of these animals have been identified by oocyst morphology alone with little or no host specificity and/or molecular data to support identification it is not known how many of the reported isolates are actually *C. parvum* or other species. Cryptosporidiosis is a cause of morbidity and mortality in animals and humans, resulting primarily in diarrhea, and resulting in the most severe infections in immune-compromised individuals. Of 15 named species of *Cryptosporidium* infectious for nonhuman vertebrate hosts *C. baileyi*, *C. canis*, *C. felis*, *C. hominis*, *C. meleagridis*, *C. muris*, and *C. parvum* have been reported to also infect humans. Humans are the primary hosts for *C. hominis*, and except for *C. parvum*, which is widespread amongst nonhuman hosts and is the most frequently reported zoonotic species, the remaining species have been reported primarily in immunocompromised humans. The oocyst stage can remain infective under cool, moist conditions for many months, especially where water temperatures in rivers, lakes, and ponds remain low but above freezing. Surveys of surface water, groundwater, estuaries, and seawater have dispelled the assumption that *Cryptosporidium* oocysts are present infrequently and in geographically isolated locations. Numerous reports of outbreaks of cryptosporidiosis related to drinking water in North America, the UK, and Japan, where detection methods are in place, indicate that water is a major vehicle for transmission of cryptosporidiosis.

Keywords: Cryptosporidiosis; Taxonomy; Epidemiology; Zoonoses; Water; Marine

* Tel.: +1 301 504 8750; fax: +1 301 504 6608.
E-mail address: rfayer@anri.barc.usda.gov.

0304-4017/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2004.09.004

Contents

1. Introduction

Cryptosporidium is a genus of protozoan parasites with species that infect fish, amphibians, reptiles, birds and mammals. Although species of *Cryptosporidium* were described at the beginning of the 20th century it was near the end of that century before they were widely recognized as pathogens of domesticated livestock and poultry, companion animals, and wildlife, and a threat to public health. Recognition that contaminated drinking and recreational water was a major source of transmission followed the discovery of the environmentally robust oocyst stage that transmitted the infection from host to host through contaminated feces. Development of molecular tools to identify morphologically indistinguishable species enabled investigators to define relationships between parasite species, potential hosts, and pathways of transmission.

For what was once thought to be the single species *Cryptosporidium parvum*, infecting a reported 155 mammalian hosts including humans, several species have now been identified associated with specific hosts and many genotypes have been identified that could acquire species status. This review identifies the species, the hosts, and the biology of the zoonotic species. Treatment and disinfection are discussed and future trends are suggested.

2. Historical time-line

In 1907, Ernest Edward Tyzzer was the first person to describe life cycle stages of a protozoan parasite he frequently found in the gastric glands of his laboratory mice and Aspores@ (oocysts) he found in the feces (Tyzzer, 1907). In 1910, reporting in greater detail, he proposed *Cryptosporidium muris* as a new genus and the type species. In 1912 he published a report naming another new species, *C. parvum*, with stages that develop only in the small intestine of mice and with oocysts smaller than those of *C. muris*. This genus remained relatively obscure for nearly half a century. In 1955 a new species, *C. meleagridis*, was reported in turkeys (Slavin, 1955), and in 1971 *Cryptosporidium* was first reported to be associated with bovine diarrhea (Panciera et al., 1971). At the time neither appeared to arouse a great deal of interest. In 1976 the first two reports of cryptosporidiosis in immune compromised humans appeared nearly simultaneously (Nime et al., 1976; Meisel et al., 1976). But it was not until 6 years later, following a report from the U.S. Centers for Disease Control of 21 men in six cities with concurrent cryptosporidiosis and AIDS (Goldfarb et al., 1982), that significant interest in cryptosporidiosis was aroused. In 1977 the first complete report of *Cryptosporidium* in reptiles was provided by Brownstein et al. (1977). Subsequently, reports describing cryptosporidiosis in a wide range of mammals, birds, and reptiles began to appear. Identification of species was based primarily on oocyst size and shape and host susceptibility. In the 1990s, the application of molecular techniques to the identification of isolates brought both clarification and complexity to our understanding of species of *Cryptosporidium* and host specificity. Although outbreaks associated with drinking water and recreational water were reported from the late 1980s to present, the defining recognition of *Cryptosporidium* as a public health problem came in 1993 with the world's largest recorded water-borne disease outbreak—the outbreak of cryptosporidiosis associated with drinking water in Milwaukee, Wisconsin (MacKenzie et al., 1994).

3. Taxonomy and phylogeny

The taxonomic status of *Cryptosporidium* and the naming of species is undergoing rapid change as new information, primarily supported by molecular data, is published almost monthly. Members of this protozoan genus in the phylum Apicomplexa were long thought to be closely related to the coccidia, important parasites in human and veterinary medicine. Despite strong morphological similarities to the coccidia throughout the life cycle and the presence of mitochondrion-specific genes (Riordan et al., 1999), it has not been shown that *C. parvum* possesses a mitochondria-like organelle (Tetley et al., 1998) as found in

Table 1
Species of *Cryptosporidium*

Species name	Type hosts	References
Cryptosporidium andersoni	Bovine (*Bos taurus*)	Lindsay et al. (2000)
Cryptosporidium baileyi	Chicken (*Gallus gallus*)	Current et al. (1986)
Cryptosporidium canis	Dog (*Canis familiaris*)	Fayer et al. (2001)
Cryptosporidium felis	Cat (*Felis catis*)	Iseki (1979)
Cryptosporidium galli	Birds (Spermestidae), birds (Fringillidae), birds (*G. gallus*), birds (*Tetrao urogallus*), birds (*Pinicola enucleator*)	Revised by Ryan et al. (2003)
Cryptosporidium hominis	Human (*Homo sapiens*)	Morgan-Ryan et al. (2002)
Cryptosporidium meleagridis	Turkey (*Meleagris gallopavo*)	Slavin (1955)
Cryptosporidium molnari	Fish (*Sparus aurata*), fish (*Dicentrarchus labrax*)	Alvarez-Pellitero and Sitja-Bobadilla (2002)
Cryptosporidium muris	Mouse (*Mus musculus*)	Tyzzer (1910)
Cryptosporidium nasorum	Fish (*Naso literatus*)	Hoover et al. (1981)
Cryptosporidium parvum	Mouse (*M. musculus*)	Tyzzer (1912)
Cryptosporidium saurophilum	Lizards	Koudela and Modry (1998)
Cryptosporidium serpentis	Snakes (*Elaphe guttata*), snakes (*E. subocularlis*), snakes (*Sanzinia madagascarensis*)	Levine (1980)
Cryptosporidium varanii	Emerald monitor lizard	Pavlasek et al. (1995)
Cryptosporidium wrairi	Guinea pig (*Cavia porcellus*)	Vetterling et al. (1971)

classical coccidia. Molecular data suggest that *Cryptosporidium* may be more closely related to gregarines (Carreno et al., 1999) and the finding of life cycle stages similar to those of gregarines supports this suggestion (Hijjawi et al., 2002). Some molecular data also suggest an ancestral relationship to *Helicobacte*r bacteria (Striepen et al., 2002).

The presently recognized 15 species of *Cryptosporidium* and their type hosts are listed in Table 1. An in-depth review of each species has recently appeared (Xiao et al., 2004). Although *C. parvum* appears to be the most widespread species, reported in over 150 mammalian hosts (Fayer et al., 2000a), most reports were based only on observations of oocyst morphology. This trait, in the absence of biological and molecular data, is now regarded as insufficient to identify species (Fayer et al., 2000a). Morphologically identical subtypes of *C. parvum*, including the deer, ferret, marsupial, monkey, and pig types (Xiao et al., 1999), could eventually be recognized as separate species, as the human genotype (*C. parvum* type 1) became recognized as *C. hominis* (Morgan-Ryan et al., 2002) and the dog genotype became recognized as *C. canis* (Fayer et al., 2001).

4. Host specificity

Most species of *Cryptosporidium* appear to have some host specificity but are not strictly host specific. For example, *C. parvum* (previously Type 2), apparently the least host specific species, has been identified in mice, cattle, humans, horses, and many other

mammalian hosts. Others, including *C. baileyi*, *C. canis*, *C. felis*, *C. meleagridis*, and *C. muris*, once thought to be host specific for chickens, dogs, cats, turkeys, and mice, respectively, have all been found to infect humans and therefore must also be considered zoonotic (Ditrich et al., 1991; Pedraza-Diaz et al., 2000, 2001; Guyot et al., 2001; Xiao et al., 2001; Caccio et al., 2002). An isolate of *C. hominis* (previously *C. parvum* Type 1), once thought to infect only humans, has been found in nature to infect the dugong, a marine mammal (Morgan et al., 2000) and therefore is also zoonotic. Furthermore, under laboratory conditions *C. hominis* was found to infect other animals including a lamb, gnotobiotic pigs, and calves, but not immunosuppressed mice (Widmer et al., 2000; Giles et al., 2001; Pereira et al., 2002; Akiyoshi et al., 2002). These examples illustrate the complexity of attempting to use host specificity as a trait for determining species, and the likely fallibility of suggesting the host range of a known species.

5. Life Cycle, infectious dose, and patency

All species of *Cryptosporidium* are obligate intracellular parasites. The endogenous stages of *C. parvum* have been described in greater detail than those of other species (O'Donoghue, 1995; Fayer et al., 1997). The only stage found outside the host is the oocyst stage. Oocyst ID_{50} values are low. For human volunteers that received three different isolates of *C. parvum* oocysts, the ID_{50} ranged from 9 to 1024 oocysts (Okhuysen et al., 1999). Preweaned ruminants appear especially susceptible to infection. Some neonates taken at birth and immediately delivered to clean rooms began excreting oocysts 3 days later, suggesting susceptibility to an extremely low exposure dose or in utero transmission. After the oocyst is ingested by a suitable host the contents excyst, releasing four motile sporozoites that invade and parasitize epithelial cells primarily in the gastrointestinal tract (and rarely in extraintestinal tissues). Subsequent developmental stages are intracellular but extra-cytoplasmic, usually found at the microvillar surface of epithelial host cells. A recent report by Hijjawi et al. (2002) indicates that gregarine-like extracellular stages were observed around 72 h after experimental infection of mice, but their exact role in the life cycle is not clear. The prepatent period, the time from ingestion of infective oocysts to excretion of oocysts following completion of the life cycle, can be completed in as few as 3–5 days or can take as long as 2 weeks. In immunocompetent persons or animals the patent period, the time over which oocysts are excreted, can last for 1 to several weeks with oocysts detected only intermittently during the later days. Immunocompromised individuals experience chronic, long-term infection, often lasting several months. The number of oocysts excreted by an infected individual can vary greatly. In experiments, calves infected with 10^5 oocysts often excrete 10^9 to 10^{10} oocysts over a period of 7–10 days.

6. Survival in the environment

The oocyst stage, a tiny spore-like body consisting of four banana-shaped sporozoites surrounded by a tough protective wall can remain infective under cool, moist conditions for

many months, especially in northern climates where water temperatures in rivers, lakes, and ponds remain low but above freezing. Laboratory studies have attempted to assess the effects of cold, heat, ultraviolet radiation, and dessication on the survival of oocysts (see also Section 11). For example, oocysts of *C. parvum* suspended in deionized water at 0, 5, 10, 15, and 20 °C remained infective for mice after 6 months of storage; those held at 25 and 30 °C produced infections after 3 months; those held at 35 °C remained infectious for only 1 week (Fayer et al., 1998b). Jenkins et al. (2003) found that oocysts held in sterile water at 15 °C remained infectious for mice and cell cultures for 7 months.

Oocysts also survived in seawater for long periods of time. They remained viable for over 35 days at 4 °C in marine water held in the dark (Robertson et al., 1992). Infections in mice were highest at the lowest salinity and shortest storage time, but even under the most severe conditions of 35% salinity at 18 °C some oocysts remained infectious for 40 days (Freire-Santos et al., 1999). Another study found that oocysts held for 12 weeks at 10 °C at 10, 20, and 30% salinity were infectious for mice, as were those held at 20 °C at 10% salinity for 12 weeks, 20% for 4 weeks, and 30% for 2 weeks (Fayer et al., 1998a).

Survival in the environment also is affected by ambient moisture. Dessication has long been known to kill oocysts of *Eimeria* species. Robertson et al. (1992) found only 3% of *C. parvum* oocysts dried on microscope slides at room temperature for 2 h remained viable and slightly longer periods resulted in 100% death.

Several other factors could affect the fate of water-borne oocysts. The level of heterotrophic bacteria in natural waters was found to influence oocyst survival (Heisz et al., 1997). Survival of oocysts was greater in membrane filtered river water than in unfiltered water (Chauret et al., 1998). Another study demonstrated degradation of oocysts in the presence of *Serratia marcescens*, a bacterium with high chitinolytic activity (Zuckerman et al., 1997). Several species of rotifers, found worldwide in moist environments from soil, to puddles, ponds, rivers, and lakes, were found to ingest oocysts, but the ultimate fate and survival of the oocysts was not determined (Fayer et al., 2000b). Oocysts of *C. parvum* recovered from shellfish in experimentally contaminated saltwater aquaria were infectious for mice over 30 days after exposure (Freire-Santos et al., 2002). Histologic examination of rainbow trout in tanks contaminated with oocysts of *C. parvum* revealed 5–7 μm spherical structures deep within the epithelium of the gastrointestinal tract that stained positively with anti-*C. parvum*-specific antibody (Freire-Santos et al., 1998). The authors hypothesized that *C. parvum* either developed deep in the mucosa of fish, instead of within the microvillar border of epithelial cells (as in mammalian hosts), or that the ingested oocysts were simply retained in this location.

Oocysts come in contact with many types of soil. One study found that the proportions of potentially infective oocysts of *C. parvum* exposed to soil and bovine waste pile materials at sites on three farms decreased more rapidly than controls exposed to buffer or water held at the same temperatures (Jenkins et al., 1999). Another study in which oocysts were exposed to three soil types and held at −10 °C for one to nine freeze-thaw cycles, 99% of oocysts became inactivated within 50 days whether or not freeze-thaw cycles occurred (Kato et al., 2002). Soil samples collected from 37 farms in New York state and analyzed for the presence of *Cryptosporidium* oocysts and pH revealed fewer oocysts in soils of neutral and basic pH than in those of low pH (Barwick et al., 2003).

7. Water quality and water-borne events

There have been over 660 publications on water-borne *Cryptosporidium* from 1990 to present related to detection techniques, survival, prevalence, outbreaks, risk assessment, disinfection chemicals and strategies, and mathematical modeling. For excellent reviews see Rose et al. (1997, 2002). Surveys of surface water conducted in North America have dispelled the assumption that *Cryptosporidium* oocysts are present infrequently and in geographically isolated locations (Rose et al., 1997). Oocysts have been found in 4–100% of surface waters samples examined with concentrations ranging from 0.1 to 10,000 oocysts/100 l (Lisle and Rose, 1995). Groundwater is also impacted. Hancock et al. (1998) found that 9.5–22% of U.S. groundwater samples tested positive for *Cryptosporidium*.

Agricultural animal waste has become a national threat to U.S. water quality because runoff following rainfall events carries nutrients and pathogens into surface waters. The estimated annual manure production from cattle alone in the U.S. in 1997 was 1.2 billion tons (Anonymous, 1997). Because nearly all preweaned calves become infected and excrete large numbers of oocysts in their feces, and older cattle continue to excrete oocysts, albeit in smaller numbers per gram of feces, cattle are believed to be a major source of water-borne *Cryptosporidium*. Although hogs produce an additional 116.7 million tons of manure annually (Anonymous, 1997), the prevalence of *Cryptosporidium* infections in hogs is much less than in cattle.

Despite apparent widespread infection of wild mammals with *Cryptosporidium* (Table 2), data documenting the extent of their contribution to pollution of surface waters are lacking.

7.1. Drinking water

Approximately 49 drinking water related outbreaks due to *Cryptosporidium* were reported between 1984 and 1999, mostly in North America, the UK, and Japan (Fayer et al., 2000a) where detection and monitoring systems were in place. The larger outbreaks in Texas, Georgia, Oregon, Ontario, British Columbia, British Columbia, Japan, and the UK affected an estimated 2006, 12,960, 15,000, >1000, 14,500, 2097, >9000, and >4321 persons, respectively (Fayer et al., 2000a). But the largest water-borne disease outbreak ever recorded for any pathogen, resulted in cryptosporidiosis in approximately 403,000 persons in Milwaukee, Wisconsin in the spring of 1993, due to *Cryptosporidium* with no species identified (MacKenzie et al., 1994). Based on death certificate records for 2 years following the outbreak, cryptosporidiosis-associated deaths were reported for 54 residents (Hoxie et al., 1997). Runoff from dairy farms, drainage from an abattoir, and other sources were suspected. Later molecular analysis of stored fecal specimens from the outbreak identified oocyst derived DNA identical to the *Cryptosporidium* human genotype (Peng et al., 1997), suggesting the source of the organisms was from human feces. This genotype is now recognized as a separate species—*C. hominis* (Morgan-Ryan et al., 2002). Virtually all outbreaks for which a cause could be determined were due to deficiencies at water treatment plants. Rainfall and runoff events are major factors affecting the presence of total microbial load, including *Cryptosporidium*, in surface waters and drinking water reservoirs

Table 2
C. parvum (and *C. parvum*-like) checklist of 155 mammalian hosts (modified and updated from Fayer et al., 2000a)

Order: Artiodactyla
- *Addax nasomaculatus* (Addax)
- *Aepyceros melampus* (Impala)
- *Ammotragus lervia* (Barbary sheep)
- *Antidorcas marsupialis* (Springbok)
- *Antilope cervicapra* (Blackbuck)
- *Axis axis* (Axis deer)
- *Bison bison* (American bison)
- *Bison bonasus* (European bison)
- *Bos indicus* (Zebu)
- *B. taurus* (Ox)
- *Boselaphus tragocamelus* (Nilgai)
- *Bubalus bubalis* (Water buffalo)
- *Bubalus depressicornis* (Lowland anoa)
- *Camelus bactrianus* (Bactrian camel)
- *Capra falconeri* (Turkomen markhor)
- *Capra hircus* (Goat)
- *Capreolus capreolus* (Roe deer)
- *Cervus albirostris* (Thorold's deer)
- *Cervus duvauceli* (Barasingha deer)
- *Cervus elaphus* (Red deer/elk/wapiti)
- *Cervus eldi* (Eld's deer)
- *Cervus nippon* (Sika deer)
- *Cervus unicolor* (Sambar)
- *Connochaetes gnou* (Wildebeest)
- *Connochaetes taurinus* (Blue-beared gnu)
- *Dama dama* (Fallow deer)
- *Elaphurus davidianus* (Pere David's deer)
- *Gazella dama* (Addra gazelle)
- *Gazella dorcas* (Dorca's gazelle)
- *Gazella leptoceros* (Slender-horned gazelle)
- *Gazella subgutterosa* (Persian gazelle)
- *Gazella thomsoni* (Thomson's gazelle)
- *Giraffa camelopardalis* (Giraffe)
- *Hexaprotodom liberiensis* (Pygmy hippopatomus)
- *Hippotragus niger* (Sable antelope)
- *Kobus ellipsiprymmus* (Ellipsen waterbuck)
- *Lama glama* (Llama)
- *Lama guanicoe* (Guanaco)
- *Lama pacos* (Alpaca)
- *Muntiacus reevesi* (Muntjac deer)
- *Odocoileus hemionus* (Mule deer)
- *Odocoileus virginianus* (White-tailed deer)
- *Oryx gazella callotys* (Fringe-eared oryx)
- *Oryx gazella dammah* (Scimitar-horned oryx)
- *Ovis aries* (Sheep)
- *Ovis musimon* (Mouflon)
- *Ovis orientalis* (Urial)

Table 2 (*Continued*)

Sus scrofa (Pig)
Syncerus caffer (African buffalo)
Taurotragus oryx (Eland)
Tayassu tajacu (Collared peccary)
Tragelaphus eurycerus (Bongo)

Order: Carnivora
Acironyx jubatus (Cheetah)
C. familiaris (Dog)
Canis latrans (Coyote)
Felis catus (Cat)
Helarctos malayanus (Malayan bear)
Martes foina (Beech marten)
Meles meles (Badger)
Mephitis mephitis (Striped skunk)
Mustela putorius (Ferret)
Panthera pardus (Leopard)
Procyon lotor (Raccoon)
Urocyon cinereoargenteus (Grey fox)
Ursus americanus (Black bear)
Ursus arctos (Brown bear)
Ursus (*Thalarctos*) *maritimus* (Polar bear)
Vulpes vulpes (Red fox)
Zalophus californianus (California sea lion)

Order: Chiroptera
Eptesicus fuscus (Big brown bat)
Myotis adversus (Large-footed mouse-eared bat)

Order: Insectivora
Ateletrix albiventris (African hedgehog)
Crocidura russula (Greater white-toothed shrew)
Erinaceus europaeus (European hedgehog)
Sorex araneus (Long-tailed shrew)
Sorex minutus (Pygmy shrew)

Order: Lagomorpha
Oryctolagus cuniculus (Rabbit)
Sylvilagus floridanus (Cottontail)

Order: Marsupialia
Antechinus stuartii (Brown antechinus)
Didelphis virginiana (Opossum)
Isodon obesulus (Southern brown bandicoot)
Macropus giganteus (Eastern grey kangaroo)
Macropus rufogriseus (Red neck wallaby)
Macropus rufus (Red kangaroo)
Phascolarctos cinereus (Koala)
Thylogale billardierii (Pademelon)
Trichosurus vulpecula (Brushtail possum)

Table 2 (*Continued*)

Order: Perissodactyla
- *Ceratotherium simum* (Southern white rhinoceros)
- *Equus caballus* (Horse)
- *Equus przewalski* (Miniature horse)
- *Equus zebra* (Zebra)
- *Rhinoceros unicornis* (Rhinoceros)
- *Tapirus terrestris* (Brazilian tapir)

Order: Primates
- *Ateles belzebuth* (Marimonda spider monkey)
- *Calithrix jacchus* (Common marmoset)
- *Cercocebus albigena* (Mangabey)
- *Cercocebus torquatus* (White-collared monkey)
- *Cercopithecus aethiops* (Velvet monkey)
- *Cercopithecus campbelli* (Campbell's mona)
- *Cercopithecus talapoin* (Talapoin monkey)
- *Erythrocebus patas* (Patas monkey)
- *Eulemur macaco* (Black lemur)
- *Gorilla gorilla* (Gorilla)
- *H. sapiens* (Humans)
- *Hylobates syndactylus syndactylus* (Siamang)
- *Lemur catta* (Ring-tailed lemur)
- *Lemur macacomayottensis* (Brown lemur)
- *Lemur variegatus* (Ruffed lemur)
- *Macaca fascicularis* (Long-tailed macaque)
- *Macaca fuscata* (Japanese macaque)
- *Macaca mulatta* (Rhesus monkey)
- *Macaca nemestrina* (Cotton-tipped/pigtail macaque)
- *Macaca radiata* (Bonnet macaque)
- *Macaca thibetana* (Pere David's macaque)
- *Mandrillus leucophaeus* (Drill)
- *Nycticebus pygmaeus* (Lesser slow loris)
- *Papio anubis* (Olive baboon)
- *Papio cynocephalus* (Baboon)
- *Pithecia pithecia* (White-faced saki)
- *Pongo pygmaeus* (Orangutan)
- *Saguinus oedipus* (Cotton-topped tamarin)
- *Saimiri sciureus* (Squirrel monkey)
- *Varecia variegata* (Red-ruffed lemur)

Order: Rodentia
- *Apodemus agrarius* (Field mouse)
- *Apodemus flavicollis* (Field mouse)
- *Apodemus sylvaticus* (Field mouse)
- *Castor canadensis* (Beaver)
- *Castor fiber* (European beaver)
- *C. porcellus* (Guinea pig)
- *Chinchilla laniger* (Chinchilla)
- *Clethrionomys glareolus* (Red-backed vole)
- *Geomys bursarius* (Pocket gopher)

Table 2 (*Continued*)

Glaucomys volans (Flying squirrel)
Hystrix indica (Indian porcupine)
Marmota monax (Woodchuck)
Mesocricetus auratus (Golden hamster)
Microtus agrestis (Field vole)
Microtus arvalis (Orkney vole)
M. musculus (House mouse)
Mus spretus (Western Mediterranean mouse)
Myocastor coypus (Coypu)
Ondatra zibethicus (Muskrat)
Rattus norvegicus (Norwegian rat)
Rattus rattus (house rat)
Sciurus carolinensis (Gray squirrel)
Sciurus niger (Fox squirrel)
Sigmodon hispidus (Cotton rat)
Spermophilus beecheyi (California ground squirrel)
Spermophilus tridecemlineatus (13-lined ground squirrel)
Tamias sibiricus (Siberian chipmunk)
Tamias striatus (Chipmunk)
Order: Monotremata
Tacyglossus aculeatus (Echidna)
Order: Proboscidea
Elephas maximus (Indian elephant)
Loxodonta africana (African elephant)
Order: Sirenia
Dugong dugon (Dugong)

(Kistemann et al., 2002). In one study of source water supplies to 66 water treatment plants in 14 states and 1 Canadian province, 87.1% of the samples were positive for *Cryptosporidium* oocysts with densities of 0.07–484 oocysts per liter (LeChevallier et al., 1991).

7.2. Recreational water

Swimming is one of the most popular recreational activities worldwide with over 350 million person-events estimated to take place annually in the USA alone. In the past few years over 10,000 people were identified in 31 locations as acquiring cryptosporid-iosis from recreational waters (Fayer et al., 2000a). In public pools, the combination of frequent fecal contamination, oocyst resistance to chlorine, low infectious dose, and high bather densities facilitate transmission. Even optimal conditions of pool design, water quality, filtration, and disinfection cannot prevent fecal accidents. However, the likelihood for water-borne transmission increases when recreational waters are used by diapered children, toddlers, and incontinent persons. To reduce transmission in recreational waters pool operators, public health officials, and users must work together

to develop plans for improving filtration and turnover rates, and separating plumbing/filtration for high-risk kiddie pools. They should identify specific response actions to fecal accidents, require barrier garments such as swim diapers, and educate patrons and staff. Simple prevention measures such as refraining from water related recreational a activities during episodes of diarrhea, refraining from swallowing recreational water, using good diaper changing and hand washing practices, supervising frequent toilet breaks for young children, and promoting showering to remove fecal residue before pool use, will reduce transmission. When rivers, lakes, and marine environments are used for recreation, contamination from uncontrollable animal and human sources add to the fecal burden making the aforementioned safeguards difficult or impossible to implement.

7.3. Estuarine and marine waters

Shellfish filter enormous quantities of water removing small particles for food. Several reports have indicated the presence of oocysts in oysters in estuarine tributaries of Chesapeake Bay (Fayer et al., 1998a, 1999, 2002). By molecular methods, most oocysts were found to be *C. parvum* and to a lesser extent *C. hominis*, *C. baileyi* and *C. canis*. The highest percentage of contaminated shellfish identified in a 3-year study were found shortly after the greatest rainfall event during that time period, suggesting runoff as the most likely source (Fayer et al., 2002). Oocysts of *C. parvum* have also been recovered from oysters, clams mussels, and cockles in coastal waters of Ireland, Spain, and the United States (Chalmers et al., 1997b; Freire-Santos et al., 2000; Gomez-Bautista et al., 2000; Fayer et al., 2003). Oocysts also find their way to coastal waters used for recreation. *Cryptosporidium* oocysts were recovered in marine water near a sewage outfall, canals impacted by runoff, and bathing beaches off the coast of Honolulu, Hawaii (Johnson et al., 1995).

8. Distribution and prevalence

Oocysts are transmitted from an infected host to susceptible hosts. Transmission can be human-to-human, animal-to-animal, human-to-animal, animal-to-human, water-borne, foodborne, and possibly airborne.

8.1. Human

Cryptosporidium infection has been reported in persons from 3 days of age to 95 years of age, but data suggest that young children are most susceptible to infection. A report of widespread human infection first appeared in 1982, related to men in the United States with the newly emergent disease—acquired immune deficiency syndrome or AIDS (Goldfarb et al., 1982). Within 2 years it became apparent that another group at risk were children in day-care centers (Anonymous, 1984). By 1986 the U.S. Centers for Disease Control reported that 3.6% of 19,817 AIDS cases had cryptosporidiosis and that their fatality rate was 61%.

The most thorough review of geographic distribution and prevalence based on oocyst detection and on seroprevalence studies in humans was compiled by Ungar (1990). More than 100 geographically based surveys from 40 countries were reviewed. Based on detection of oocysts in fecal specimens, the prevalence of human infection in African countries (2.6–21.3%), Central and South American countries (3.2–31.5%), Asia countries (1.3–13.1%) and others in the Pacific and Caribbean areas, is greater than that in Europe (0.1–14.1%) or North America (0.3–4.3%). Several more recent surveys have reported data from other geographic locations but the trend remains the same. Better sanitation and cleaner drinking water in the more industrialized countries most likely accounts for this difference.

8.2. Animal

A list of the 155 species of mammals reported to be infected with *C. parvum* or *C. parvum*-like organisms appears in Table 2. Most infected animals fall within the Orders Artiodactyla, Primates, and Rodentia. *Cryptosporidium* from most of these host species have been identified by microscopy using morphologic features alone. Host specificity and/or molecular data are available for very few isolates from these animals. Therefore, it is not well understood how many of the reported isolates are actually *C. parvum* or other species that might be infectious for humans or other animals. Several groups of investigators have clearly shown the need for molecular data as a basis for identifying isolates.

Domesticated cattle are the most thoroughly documented host species with respect to distribution and prevalence of *Cryptosporidium* infection. Cattle worldwide are infected with *C. parvum*. The highest prevalence has been identified in pre-weaned calves. They are the most vulnerable to morbidity from cryptosporidiosis and they produce the greatest number of oocysts that contaminate the environment. Similar findings have been reported for other young ruminants such as sheep, goats, and deer, and exotic ruminants in wildlife parks (Angus, 1990). A close association between livestock and feral rodents (Klesius et al., 1986; Chalmers et al., 1997a) facilitates transmission of *C. parvum* between these hosts and ensures its perpetuation.

9. Clinical features

Asymptomatic infections have been reported. For others, the clinical course and severity of infection can vary considerably from person to person, depending in large part on the immune status of the host. The most noteworthy symptom in immunologically healthy persons is diarrhea, usually voluminous and watery. Mucous is sometimes present but blood and leukocytes are rare. Abdominal discomfort, anorexia, nausea, vomiting, weight loss, fever, fatigue and respiratory problems may accompany diarrhea. In 50 immunologically healthy persons, the mean duration of illness was 12 days and ranged from 2 to 26 days (Jokipii and Jokipii, 1986). After cessation of symptoms 19% of patients had positive stools for a mean period of 6.9 days. Parasites are generally restricted to the lower small intestine.

In persons with immune deficiencies related to malnutrition, viral infections such as measles and human immunodeficiency virus (HIV), and exogenous immunotherapy for cancer or other diseases, the duration and severity of illness depends on the extent of cellular immunity impairment. In general, as CD4 T cell numbers or function decreases, the severity of illness increases. Infections can become chronic and life threatening with frequent voluminous watery stools leading to dehydration. In severe cases life cycle stages have been seen in cells in the respiratory tree, liver, gall bladder, pancreas, and other extraintestinal sites. With the application of highly active anti-retroviral therapy (HAART), a cocktail of anti-viral drugs, the severity and number of cases of cryptosporidiosis in HIV infected persons decreased although no specific medication for treatment of cryptosporidiosis has been approved by the U.S. Federal Drug Administration.

Livestock and companion animals exhibit the same prominent clinical sign of infection as humans, watery diarrhea. Clinical signs have been reported for cattle, sheep, goats, and farm-raised deer (Angus, 1990). Severe cases result in mortality. Oocysts of multiple species and genotypes have been identified in feces of pigs, horses, dogs, and cats, but clinical signs are rare.

The molecular basis for pathogenicity is not understood and no specific virulence factors have been unequivocally shown to cause direct or indirect damage to host tissues (Okhuysen and Chappell, 2002). Loss of absorptive epithelium including apoptosis and villus atrophy results in malabsorption, and release of inflammatory cell mediators stimulate electrolyte secretion and diarrhea (Gookin et al., 2002). In a piglet model Argenzio et al. (1996) found that prostaglandins altered NaCl transport primarily be stimulating the enteric nervous system. Because of the profuse secretory diarrhea experienced by some patients, it has been hypothesized that *C. parvum* produces an enterotoxin.

10. Prophylaxis and therapy

After many years of research on strategies and drugs for treatment and control of cryptosporidiosis, there are no consistently effective, approved products for either animals or humans. Some have proven toxic at doses required to reduce parasite multiplication, others have shown some efficacy only in animal models, and most others have shown no efficacy. Paromomycin, which provided prophylaxis for experimentally infected calves (Fayer and Ellis, 1993), was inconsistently helpful for humans; some patients continued to excrete oocysts and others relapsed when therapy was removed (Blagburn and Soave, 1997). Nitazoxanide, a nitrothiazole benzamide with a wide spectrum of activity against bacteria, protozoa, and helminths, has shown efficacy in early trials with human subjects (Blagburn and Soave, 1997). However, as Amadi et al. (2002) reported, a 3-day course of nitazoxanide significantly improved the resolution of diarrhea and parasite eradication and mortality in HIV-seronegative but not in HIV-seropositive patients. Similar findings have been reported for other drugs, suggesting that an immunological component is ultimately necessary for recovery.

Phylogenetic studies of *Cryptosporidium* may help identify related organisms for which prophylaxis and therapy have been developed. For example, Striepen et al. (2002) found

that the *C. parvum* gene for the enzyme inosine 5-monophosphate-dehydrogenase was very similar to that of *Helicobacter pylori*, a surprising relationship that provides the opportunity to test or develop new drugs.

Persistent infections and increased susceptibility to infection in animals and persons with reduced cellular immunity and depleted IL-12 and IFN-γ provided clues to their potential importance for control of *Cryptosporidium* infection (Riggs, 1997). Hyperimmune bovine colostrum, high in anti-*Cryptosporidium* antibodies has been tested for efficacy in mice, calves, and humans with mixed success (Riggs, 1997). Despite knowing the importance of cellular immunity and many attempts to identify antigens that will stimulate a protective immune response, no immunotherapeutics or vaccines are presently approved for prevention or treatment of cryptosporidiosis in animals or humans.

11. Disinfection

Many chemical disinfectants have been tested for efficacy against oocysts of *C. parvum* with mixed success (Fayer et al., 1997). The most effective, and most toxic, have been small molecular weight compounds including ammonia, ethylene oxide, methyl bromide, and ozone. Perhaps only ozone has practical use.

Chlorine, effective against many microorganisms is slow to affect *Cryptosporidium* even at high concentrations so that chlorine used under conditions in water treatment facilities and swimming pools has little or no impact on oocyst viability (Carpenter et al., 1999). Likewise, alum floccing as used by the water industry was found to have no impact on oocyst viability and high concentrations of lime or ferric sulfate were found to significantly reduce oocyst viability only at high pH levels over prolonged time (Robertson et al., 1992). Many studies have been conducted on disinfection and filtration strategies for drinking water (Rose et al., 1997). Ozone and ultraviolet light each has shown efficacy in rendering oocysts noninfectious and have applicability for disinfection of *Cryptosporidium* oocysts in water treatment facilities (Clancy et al., 2000; Kanjo et al., 2000; Rose et al., 1997). Treatment with multiple chemicals such as chlorine and monochloramine or ozone and monochloramine has demonstrated greater disinfection than exposure to a single chemical.

Although oocysts can survive for months at cold temperatures those frozen at −70 °C for 1 h or more failed to infect mice; those frozen at −20 °C for 8 but not 24 h infected mice; and those frozen at −10 °C for as long as 168 h still infected mice (Fayer and Nerad, 1996). As temperatures increase above 5 °C survival time decreases. All vials of oocysts held for 1 min at 54.4, 59.9, and 67.5 °C were infectious for mice but those held at 72.4 °C or higher for 1 min were not infectious (Fayer, 1994). Likewise, oocysts in vials held for 5 min at 59.7 °C were infectious for one of three mice but those held at 64.2 °C for 5 min were no longer infectious (Fayer, 1994). Fujima et al. (2002) found oocysts of *C. parvum*, *C. muris* and a species of chicken *Cryptosporidium* more heat sensitive, killing oocysts exposed to 55 °C after only 15–30 s. Using bench scale commercial pasteurization equipment Harp et al. (1996) found that oocysts of *C. parvum* suspended in water and milk failed to infect mice after exposure to 71.7 °C for only 5 s.

12. Future trends

At the most basic level, the process of sequencing the genome of *C. parvum* and portions of *C. hominis* is now underway. When their complete sequences are known, our understanding of the many proteins encoded by these genes will still be incomplete. Studies to understand the complex biological systems involving these proteins will follow. Proteomics, the qualitative and quantitative comparison of proteomes under different conditions, will help to further unravel biological processes and complement physical genomic research. Application will be found in the development of new drugs because most biological processes are protein driven and the majority of drugs on the market are directed against protein targets.

Laboratory application of molecular techniques to taxonomy and epidemiology is helping to characterize new and existing species and thereby differentiate field isolates of *Cryptosporidium* and determine the possible sources of the parasites. Continued application of these techniques will further aid the identification of new species and subspecies, facilitating the identification of sources of water-borne cryptosporidiosis.

Manure management programs to reduce runoff from agricultural sites, testing and repair or elimination of leaky septic tanks, and improved cleaning of waste-water that is released into surface waters will reduce loading of all water-borne pathogens including *Cryptosporidium*.

Newly designed filtration equipment and improvements in disinfection methods are underway. Application of these technologies including use of ultraviolet light, ozone and possibly other disinfection schemes will improve water quality and safety.

References

Akiyoshi, D.E., Feng, X., Buckholt, M.A., Widmer, G., Tzipori, S., 2002. Genetic analysis of a *Cryptosporidium parvum* human genotype 1 isolate passed through different hosts. Infect. Immun. 70, 5670–5675.

Alvarez-Pellitero, P., Sitja-Bobadilla, A., 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. Int. J. Parasitol. 32, 1007–1021.

Amadi, B., Mwiya, M., Musuku, J., Watuka, A., Sianongo, S., Ayoub, A., Kelly, P., 2002. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. Lancet 360, 1375–1380.

Angus, K.W., 1990. Cryptosporidiosis in ruminants. In: Dubey, J.P., Speer, C.A., Fayer, R. (Eds.), Cryptosporidiosis in Man and Animals. CRC Press, Boca Raton, FL, pp. 83–103.

Anonymous, 1984. Cryptosporidiosis among children attending day-care-centers—Georgia, Pennsylvania, Michigan, California, New Mexico. Morbid. Mortal. Weekly Rep. 33, 599–601.

Anonymous, 1997. Animal waste pollution in America: an emerging national problem. Report compiled by the minority staff of the United States Senate Committee on Agriculture, Nutritiion, and Forestry for Senator Tom Harkin, 25 pp. http://www.senate.gov/~agriculture/animalw.htm.

Argenzio, R.A., Armstrong, M., Rhoads, J.M., 1996. Role of the enteric nervous system in piglet cryptosporidiosis. J. Pharmacol. Exp. Ther. 279, 1109–1115.

Barwick, R.S., Mohammed, H.O., White, M.E., Bryant, R.B., 2003. Factors associated with the likelihood of *Giardia* spp. and *Cryptosporidium* spp. in soil from dairy farms. J. Dairy Sci. 86, 784–791.

Blagburn, B.L., Soave, R., 1997. Prophylaxis and chemotherapy: human and animal. In: Fayer, R. (Ed.), Cryptosporidium and Cryptosporidiosis. CRC Press, Boca Raton, FL, pp. 111–128.

Brownstein, D., Strandberg, J., Montali, R., Bush, M., Fortner, J., 1977. *Cryptosporidium* in snakes with hypertrophic gastritis. Vet. Pathol. 14, 606–617.

Caccio, S., Pinter, E., Fantini, R., Mezzaroma, I., Pozio, E., 2002. Human infection with *Cryptosporidium felis*: case report and literature review. Emerg. Infect. Dis. 8, 85–86.

Carpenter, C., Fayer, R., Trout, J., Beach, M., 1999. Chlorine disinfection of recreational water for *Cryptosporidium parvum*. Emerg. Infect. Dis. 5, 579–584.

Carreno, R.A., Marten, D.S., Barta, J.R., 1999. *Cryptosporidium* is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences. Parasitol. Res. 85, 899–904.

Chalmers, R.M., Sturdee, A.P., Bull, S.A., Miller, A., Wright, S.E., 1997a. The prevalence of *Cryptosporidium parvum* and *C. muris* in *Mus domesticus*, *Apodemus sylvaticus* and *Clethrionomys glareolus* in an agricultural system. Parasitol. Res. 83, 478–482.

Chalmers, R.M., Sturdee, A.P., Mellors, P., Nicholson, V., Lawler, F., Kenny, F., Timpson, P., 1997b. *Cryptosporidium parvum* in environmental samples in the Sligo area, Republic of Ireland: a preliminary report. Lett. Appl. Microbiol. 25, 380–384.

Chauret, C., Nolan, K., Chen, P., Springthorpe, S., Sattar, S.A., 1998. Aging of *Cryptosporidium parvum* oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine. Can. J. Microbiol. 44, 1154–1160.

Clancy, J.L., Bukhari, Z., Hargy, T.M., Bolton, J.R., Dussert, B.W., Marshall, M.M., 2000. Using UV to inactivate *Cryptosporidium*. J. Am. Water Works Assoc. 92, 97–104.

Current, W.L., Upton, S.J., Haynes, T.B., 1986. The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. Protozool. 33, 289–296.

Ditrich, O., Palkovic, L., Sterba, J., Prokopic, J., Loudova, J., Giboda, M., 1991. The first finding of *Cryptosporidium baileyi* in man. Parasitol. Res. 77, 44–47.

Fayer, R., 1994. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. Appl. Environ. Microbiol. 60, 2732–2735.

Fayer, R., Ellis, W., 1993. Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. J. Parasitol. 79, 771–774.

Fayer, R., Graczyk, T.K., Lewis, E.J., Trout, J.M., Farley, C.A., 1998a. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. Appl. Environ. Microbiol. 64, 1070–1074.

Fayer, R., Trout, J.M., Jenkins, M.C., 1998b. Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. J. Parasitol. 84, 1165–1169.

Fayer, R., Lewis, E.J., Trout, J.M., Graczyk, T.K., Jenkins, M.C., Higgins, J., Xiao, L., Lal, A.A., 1999. *Cryptosporidium* in oysters from commercial harvesting sites in the Chesapeake Bay. Emerg. Infect. Dis. 5, 706–710.

Fayer, R., Morgan, U., Upton, S.J., 2000a. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. Int. J. Parasitol. 30, 1305–1322.

Fayer, R., Trout, J.M., Walsh, E., Cole, R., 2000b. Rotifers ingest oocysts of *Cryptosporidium parvum*. J. Eukaryot. Microbiol. 47, 161–163.

Fayer, R., Nerad, T., 1996. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. Appl. Environ. Microbiol. 62, 1431–1433.

Fayer, R., Speer, C.A., Dubey, J.P., 1997. The general biology of Cryptosporidium. In: Fayer, R. (Ed.), Cryptosporidium and Cryptosporidiosis. CRC Press, Boca Raton, FL, pp. 1–41.

Fayer, R., Trout, J.M., Lewis, E.J., Xiao, L., Lal, A.A., Jenkins, M.C., Graczyk, T.K., 2002. Temporal variability of *Cryptosporidium* in the Chesapeake Bay. Parasitol. Res. 88, 998–1003.

Fayer, R., Trout, J.M., Lewis, E.J., Santin, M., Zhou, L., Lal, A.A., Xiao, L., 2003. Contamination of Atlantic coast commercial shellfish with *Cryptosporidium*. Parasitol. Res. 89, 141–145.

Fayer, R., Trout, J.M., Xiao, L., Morgan, U.M., Lal, A.A., Dubey, J.P., 2001. *Cryptosporidium canis* n. sp. from domestic dogs. J. Parasitol. 87, 1415–1422.

Freire-Santos, F., Oteiza-Lopez, A.M., Vergara-Castiblanco, C.A., Ares-Mazias, M.E., 1999. Effect of salinity, temperature and storage time on mouse experimental infection by *Cryptosporidium parvum*. Vet. Parasitol. 87, 1–7.

Freire-Santos, F., Gomez-Couso, H., Ortega-Inarrea, M.R., Castro-Hermida, J.A., Oteiza-Lopez, A.M., Garcia-Martin, O., Ares-Mazias, M.E., 2002. Survival of *Cryptosporidium parvum* oocysts recovered from experimentally contaminated oysters (*Ostrea edulis*) and clams (*Tapes decussatus*). Parasitol. Res. 88, 130–133.

Freire-Santos, F., Oteiza-Lopez, A.M., Vergara-Castiblanco, C.A., Ares-Mazias, M.E., Alvarez-Suarez, E., Garcia-Martin, O., 2000. Detection of *Cryptosporidium* oocysts in bivalve molluscs destined for human consumption. J. Parasitol. 86, 853–854.

Freire-Santos, F., Vergara-Castiblanco, C.A., Tojo-Rodriguez, J.L., Santamarina-Fernandez, T., Ares-Maza, E., 1998. *Cryptosporidium parvum*: an attempt at experimental infection in rainbow trout *Oncorhynchus mykiss*. J. Parasitol. 84, 935–938.

Fujima, T., Matsui, T., Kobayashi, F., Haruki, K., Yoshino, Y., Kajima, J., Tsuji, M., 2002. The effect of heating against *Cryptosporidium* oocysts. J. Vet. Med. Sci. 64, 199–200.

Goldfarb, J., Tannowitz, H., Grossner, R., Bonanno, C., Kaufman, D., Ma, P., et al., 1982. Cryptosporidiosis: assessment of chemotherapy of males with acquired immune deficiency syndrome (AIDS). Morbid. Mortal. Weekly Rep. 31, 589–591.

Giles, M., Webster, K.W., Marshall, J.A., Catchpole, J., Goddard, T.M., 2001. Experimental infection of a lamb with *Cryptosporidium parvum* type 1. Vet. Rec. 149, 523–525.

Gomez-Bautista, M., Ortega-Mora, L.M., Tabares, E., Lopes-Rodas, V., Costas, E., 2000. Detection of infectious *Cryptosporidium parvum* oocysts in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*). Appl. Environ. Microbiol. 66, 1866–1870.

Gookin, J.L., Nordone, S.K., Argenzio, R.A., 2002. Host responses to *Cryptosporidium* infection. J. Vet. Int. Med. 16, 12–21.

Guyot, K., Follet-Dumoulin, A., Lelievre, E., Sarfati, C., Rabodonirina, M., Nevez, G., Cailliez, J.C., Camus, D., Deicas, E., 2001. Molecular characterization of *Cryptosporidium* isolates obtained from humans in France. J. Clin. Microbiol. 39, 3472–3480.

Hancock, C.M., Rose, J.B., Callahan, M., 1998. Crypto and Giardia in US groundwater. J. Am. Water Works Assoc. 90, 58–61.

Harp, J.A., Fayer, R., Pesch, B.A., Jackson, G.J., 1996. Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. Appl. Environ. Microbiol. 62, 2866–2868.

Heisz, M., Chauret, C., Chen, P., Springthorpe, S., Sattar, S.A., 1997. In vitro survival of *Cryptosporidium* oocysts in natural waters. In: Fricker, C., Clancy, J.L., Rochelle, P.A. (Eds.), Proceedings of the 1997 International Symposium on Waterborne *Cryptosporidium*. American Water Works Association, Denver, CO, pp. 171–175.

Hijjawi, N.S., Meloni, B.P., Ryan, U.M., Olsen, M.E., Thompson, R.C.A., 2002. Successful in vitro cultivation of *Cryptosporidium andersoni*: evidence for the existence of novel extracellular stages in the life cycle and implications for the classification of *Cryptosporidium*. Int. J. Parasitol. 32, 1719–1726.

Hoover, D.M., Hoerr, F.J., Carlton, W.W., Hinsman, E.J., Ferguson, H.W., 1981. Enteric cryptosporidiosis in a Naso tang, *Naso lituratus* Bloch Schneider. J. Fish. Dis. 4, 425–428.

Hoxie, N.J., Davis, J.P., Vergeron, J.M., Nashold, R.D., Blair, K.A., 1997. Cryptosporidiosis associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. Am. J. Pub. Health 87, 2032–2035.

Iseki, M., 1979. *Cryptosporidium felis* sp. n. (Protozoa: Eimeriorina) from the domestic cat. Jpn. J. Parasitol. 5, 285–307.

Johnson, D., Reynolds, K., Gerba, C., Pepper, L., Rose, J., 1995. Detection of *Giardia* and *Cryptosporidium* in marine waters. Water Sci. Technol. 31, 439–442.

Jokipii, L., Jokipii, A.M., 1986. Timing of symptoms and oocyst excretion in human cryptosporidiosis. N. Engl. J. Med. 315, 1643–1647.

Jenkins, M., Trout, J.M., Higgins, J., Dorsch, M., Veal, D., Fayer, R., 2003. Comparison of tests for viable and infectious *Cryptosporidium parvum* oocysts. Parasitol. Res. 89, 1–5.

Jenkins, M.B., Walker, M.J., Bowman, D.D., Anthony, L.V., Ghiorse, W.C., 1999. Use of a sentinel system for field measurements of *Cryptosporidium parvum* oocyst inactivation in soil and animal waste. Appl. Environ. Microbiol. 65, 1998–2005.

Kanjo, Y., Kimata, I., Iseki, M., Miyanaga, S., Okada, H., Banno, C., Matsumoto, M., Shimada, Y., 2000. Inactivation of *Cryptosporidium* spp. oocysts with ozone and ultraviolet irradiation evaluated by in vitro excystation and animal infectivity. Water Sci. Technol. 41, 119–125.

Kato, S., Jenkins, M.B., Fogarty, E.A., Bowman, D.D., 2002. Effects of freeze-thaw events on the viability of *Cryptosporidium parvum* oocysts in soil. J. Parasitol. 88, 718–722.

Kistemann, T., Claben, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V., Exner, M., 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. Appl. Environ. Microbiol. 68, 2188–2197.

Koudela, B., Modry, D., 1998. New species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) from lizards. Folia Parasitol. 43, 95–100.

Klesius, P.H., Haynes, T.B., Malo, L.K., 1986. Infectivity of *Cryptosporidium* sp. isolated from wild mice for calves and mice. J. Am. Vet. Med. Assoc. 189, 192–193.

LeChevallier, M.W., Norton, N.D., Lee, R.G., 1991. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Appl. Environ. Microbiol. 57, 2610–2616.

Levine, N.D., 1980. Some corrections of coccidian (Apicomplexa: Protozoa) nomenclature. J. Parasitol. 66, 830–834.

Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R., Blagburn, B.L., 2000. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporidiidae) from cattle, *Bos taurus*. J. Eukaryot. Microbiol. 47, 91–95.

Lisle, J.T., Rose, J.B., 1995. Cryptosporidum contamination of water in the USA and UK: a minireview. J. Water Supply Res. Technol. 44, 103–105.

MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmerczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., Davis, J.P., 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N. Engl. J. Med. 331, 161–167.

Meisel, J.L., Perera, D.R., Meligro, C., Rubin, C.E., 1976. Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. Gastroenterology 70, 1156–1160.

Morgan, U.M., Xiao, L., Hill, B.D., O'Donoghue, P., Limor, J., Lal, A.A., Thompson, R.C.A., 2000. Detection of the *Cryptosporidium parvum* "human" genotype in a dugong (*Dugong dugong*). J. Parasitol. 86, 1352–1354.

Morgan-Ryan, U., Fall, A., Ward, L.A., Hijjawi, N., Sulaiman, I., Fayer, R., Thompson, R.C.A., Olson, M., Lal, A.A., Xiao, L., 2002. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. J. Eukaryot. Microbiol. 49, 433–440.

Nime, F.A., Burek, J.D., Page, D.L., Holscher, M.A., Yardley, J.H., 1976. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. Gastroenterology 70, 592–598.

O'Donoghue, P., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. Int. J. Parasitol. 25, 139–195.

Okhuysen, P.C., Chappell, C.L., 2002. *Cryptosporidium* virulence determinants—are we there yet? Int. J. Parasitol. 32, 517–525.

Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R., DuPont, H.L., 1999. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. J. Infect. Dis. 180, 1275–1281.

Panciera, R., Thomassen, R., Gamer, F., 1971. Cryptosporidial infection in a calf. Vet. Pathol. 8, 479–484.

Pavlasek, I., Lavickova, M., Horak, P., Kral, J., Kral, B., 1995. *Cryptosporidium varanii* n. sp. (Apicomplexa: Cryptosporidiidae) in Emerald monitor (Varanus prasinus Schlegel, 1893) in captivity at Prague zoo. Gazella 22, 99–108.

Pedraza-Diaz, S., Amar, C., Iversen, A.M., Stanley, P.J., McLauchlin, J., 2001. Unusual cryptosporidium species recovered from human faeces: first description of *Cryptosporidium felis* and *Cryptosporidium* 'dog type' from patients in England. J. Med. Microbiol. 50, 293–296.

Pedraza-Diaz, S., Amar, C., McLauchlin, J., 2000. The identification and characterisation of an unusual genotype of *Cryptosporidium* from human faeces as *Cryptosporidium meleagridis*. FEMS Microbiol. Lett. 189, 189–194.

Peng, M.M., Xiao, L., Freeman, A.R., Arrowood, M.J., Escalante, A.A., Weltman, A.C., Ong, C.S.L., Mac Kenzie, W.R., Lal, A.A., Beard, C.B., 1997. Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. Emerg. Infect. Dis. 3, 567–573.

Pereira, S.J., Ramirez, N.E., Xiao, L., Ward, L.A., 2002. Pathogenesis of human and bovine *Cryptosporidium parvum* in gnotobiotic pigs. J. Infect. Dis. 186, 715–718.

Riggs, M.W., 1997. Immunology: host response and development of passive immunotherapy and vaccines. In: Fayer, R. (Ed.), Cryptosporidium and Cryptosporidiosis. CRC Press, pp. 129–162.

Riordan, C.E., Langreth, S.G., Sanchez, L.B., Kayser, O., Keithly, J.S., 1999. Preliminary evidence for a mitochondrion in *Cryptosporidium parvum*: phylogenetic and therapeutic implications. J. Eukaryot. Microbiol. 46, 52S–55S.

Robertson, L.J., Campbell, A.T., Smith, H.V., 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Appl. Environ. Microbiol. 58, 3494–3500.

Rose, J.B., Huffman, D.E., Gennaccaro, A., 2002. Risk and control of waterborne cryptosporidiosis. FEMS Microbiol. Rev. 26, 113–123.

Rose, J.B., Lisle, J.T., LeChevallier, M., 1997. Waterborne cryptosporidiosis: incidence outbreaks, and treatment strategies. In: Fayer, R. (Ed.), Cryptosporidium and Cryptosporidiosis. CRC Press, Boca Raton, FL, pp. 93–110.

Ryan, U.M., Xiao, L., Sulaiman, I.M., Monis, P., Lal, A.A., Fayer, R., Pavlasek, I., 2003. A redescription of *Cryptosporidium galli* Pavlasek 1999 (Apicomplexa: Cryptosporidiidae) from birds. J. Parasitol. 89, 809–813.

Slavin, D., 1955. *Cryptosporidium meleagridis* (sp. nov.). J. Comp. Pathol. 65, 262–270.

Striepen, B., White, M.W., Li, C., Guerini, M.N., Malik, S.B., Logsdon, J.M., Liu, C., Abrahamsen, M.S., 2002. Genetic complementation in apicomplexan parasites. Proc. Natl. Acad. Sci. 99, 6304–6309.

Tetley, L., Brown, S.M.A., McDonald, V.M., Coombs, G.H., 1998. Ultrastructural analysis of the sporozoite of *Cryptosporidium parvum*. Microbiology 144, 3249–3255.

Tyzzer, E.E., 1907. A sporozoan found in the peptic glands of the common mouse. Proc. Soc. Exp. Biol. Med. Assoc. 5, 12–13.

Tyzzer, E.E., 1910. An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. J. Med. Res. 23, 487–511.

Tyzzer, E.E., 1912. *Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. Arch. Protistenkd. 26, 394–412.

Ungar, B.L.P., 1990. Cryptosporidiosis in humans (*Homo sapiens*). In: Dubey, J.P., Speer, C.A., Fayer, R. (Eds.), Cryptosporidiosis in Man and Animals. CRC Press, Boca Raton, FL, pp. 59–84.

Vetterling, J.M., Jervis, H., Merril, T., Sprinz, H., 1971. *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. J. Protozool. 18, 243–247.

Widmer, G., Akiyoshi, D., Buckholt, M.A., Feng, X., Rich, S.M., Deary, K.M., Bowman, C.A., Xu, P., Wang, Y., Wang, X., Buck, G.A., Tzipori, S., 2000. Animal propagation and genomic survey of a genotype 1 isolate of *Cryptosporidium parvum*. Mol. Biochem. Parasitol. 108, 187–197.

Xiao, L., Bern, C., Limor, J., Sulaiman, I., Roberts, J., Checkley, W., Cabrera, L., Gilman, R.H., Lal, A.A., 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. 7, 492–497.

Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004. Cryptosporidium taxonomy: recent advances and implications for public health. Clin. Micro. Rev. 17, 72–97.

Xiao, L., Morgan, U., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C.A., Fayer, R., Lal, A.A., 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl. Environ. Microbiol. 65, 3386–3391.

Zuckerman, U., Gold, D., Shelef, G., Yuditsky, A., Arman, R., 1997. Microbial degradation of *Cryptosporidium parvum* by *Serratia marcescens* with high chitinolytic activity. In: Fricker, C., Clancy, J.L., Rochelle, P.A. (Eds.), Proceedings of the 1997 International Symposium on Waterborne *Cryptosporidium*. American Water Works Association, Denver, CO, pp. 297–304.